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Research Article

HIF-1α and VEGF Expression in Adult-type Diffuse High-Grade Astrocytoma

Febria Rizky Patikawa, Dyah Fauziah*, Willy Sandhika

ABSTRACT

Background: Gliomas stand as the prevalent primary malignant brain tumors in adults with astrocytoma being more common than oligodendroglioma. Based on isocitrate dehydrogenase (IDH) status, astrocytomas are classified as astrocytoma with mutated IDH and astrocytoma with wild-type IDH (glioblastoma). Tumor growth relies on angiogenesis, a process facilitated by key factors such as Vascular Endothelial Growth Factor (VEGF) and Hypoxia Inducible Factor-1α (HIF-1α). This study aims to investigate the VEGF and HIF-1α expression profiles in grade 4 astrocytomas, encompassing both mutated IDH and wild-type IDH. **Method**: This study was conducted on 43 formalin fixed paraffin embedded (FFPE) materials of surgical specimens from adult-type grade 4 astrocytoma. Immunohistochemistry with IDH1 R132H was carried out to determine the IDH status, followed by assessment of HIF-1α and VEGF expression using semi-quantitatively utilizing immunoreactive score (IRS), and categorized as negative, weak, moderate, and strong. **Results**: Statistical analysis revealed no disparity in HIF-1α expression between both tumor types, nor was there a difference in VEGF expression in both tumor types, yet a positive association was established between VEGF and HIF-1α expression levels in IDH mutant and wild type of grade 4 astrocytoma with moderate strength (r=0.433). **Conclusion**: HIF-1α and VEGF are positively linked, despite the IDH status, and simultaneously work to promote angiogenesis in diffuse high-grade astrocytoma.

Key words: Glioma, Astrocytoma IDH mutant grade 4, Glioblastoma IDH wild type, HIF-1α, VEGF.

Febria Rizky Patikawa, Dyah Fauziah*, Willy Sandhika

Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Academic General Hospital, Surabaya, INDONESIA.

Correspondence

Dyah Fauziah

Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Hospital, Surabaya, INDONESIA.

E-mail: dyah-f@fk.unair.ac.id.

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INTRODUCTION

Gliomas, comprising astrocytic tumors like glioblastoma, represent the predominant primary neoplasms within the brain, constituting approximately 77.5% of all gliomas.[1,2] With astrocytoma being more common than oligodendroglioma.[3] Based on isocitrate dehydrogenase (IDH) status, diffuse astrocytoma can be classified as astrocytoma with mutated IDH and astrocytoma with wild-type IDH. Despite the IDH status, grade 4 astrocytoma with mutated IDH and astrocytoma with wild-type IDH share the same morphological characteristics of microvascular proliferation and necrosis, in addition to increased cellularity and nuclear pleomorphism.[4,5] Tumor cell development and growth rely heavily on angiogenesis.[6] Increased VEGF expression is a consequence of elevated HIF-1α levels induced by hypoxia.[7,8] This study aims to investigate the VEGF and HIF-1α expression profiles in grade 4 astrocytomas, encompassing both mutated IDH and wild-type IDH.

METHODS

Research designs

This analytical observational study employed a cross-sectional approach. Forty-three formalin fixed paraffin embedded (FFPE) materials of surgical specimens from adult-type grade 4 astrocytoma were collected from the archives of the Laboratory of Anatomical Pathology, Dr. Soetomo Regional Public Hospital (RSUD Dr. Soetomo), Surabaya, spanning from January 2014 to December 2020.

Immunohistochemistry

Each FFPE materials were cut three times into 4 micron-thick sections, followed by deparaffinization, rehydration, and washing in running and distilled water. The antigen retrieval step using a decloaking chamber at 95°C is applied with a Target Retrieval Solution (TRS).[9] The tissue samples were then treated with mutant specific monoclonal antibody IDH1-R132H (clone IHC 132, dilution 1:100, Gene Text, USA), monoclonal antibody HIF-1α (clone EP1215Y, dilution 1:200, Biocare Medical, USA) and monoclonal antibody VEGF (clone EP1176Y, dilution 1:200, Biocare Medical, USA).

The IDH status was categorized into mutant type and wild type. Tumors with immunoreactivity of ≥10% were classified as mutant type, while tumors with <10% immunoreactivity were classified as wild type.[5] The immunoreactivity of HIF-1α and VEGF was assessed using the immunoreactive score (IRS). Positive tumor cell percentage was evaluated based on the following criteria: 0 = no staining, 1 = < 10% staining, 2 = 10-50% staining, and 3 = > 50% staining. Staining intensity was evaluated based on the following criteria: 0 = no staining, 1 = weak intensity, 2 = moderate intensity, and 3 = strong intensity. Calculating the IRS score involved multiplying the staining intensity score by the fraction of tumor cells displaying positive staining. An IRS score of 0 was interpreted as negative, while scores from 1-3 as weak, scores from 4-6 as moderate, and scores from 7-9 as strong.[10]

Data analysis

The Statistical Package for the Social Sciences (SPSS 25.0, Chicago, IL, USA) was employed to analyze

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the data statistically. The comparison of HIF-1α and VEGF expression between the two tumor groups was conducted using the Mann-Whitney test. This study employed Spearman's correlation test to analyze the interrelation of HIF-1α and VEGF expression in grade 4 astrocytoma with mutated IDH and wild-type IDH. Correlation results are deemed significant when the p-value is below 0.05.

RESULTS

Samples characteristics

In this study, grade 4 astrocytoma were mainly male, with a ratio of males to females of 2.07:1. The age range spanned between 19 and 63 years, with an average age of 46.81 years. Grade 4 astrocytoma were affected more often in the age group of >51 years old (39.53%). Based on the IDH status this study discovered that 27 (62.78%) cases were glioblastoma IDH wild type and 16 (37.78%) cases were astrocytoma IDH mutant (Table **1**).

IDH status

In this study, grade 4 astrocytoma IDH mutant was most commonly found among individuals aged 31 to 40 years, and astrocytoma with wild-type IDH was most common in the >50 years old age group. The characteristics of samples based on the IDH status are depicted in Table 2. This study discovered that 27 (62.78%) cases were glioblastoma IDH wild type and 16 (37.78%) cases were astrocytoma IDH mutant. In this study, grade 4 astrocytoma IDH mutant most commonly found among individuals aged 31 to 40 years, and astrocytoma with wild-type IDH was most common in the >50 years old age group.

HIF-1α expression

HIF-1α expression was predominantly observed within the nuclei of tumor cells, with 41 cases demonstrating its presence. Weak expression was noted in a majority of samples, accounting for 69.76% of the total cases. The negative expression of HIF-1α was found in 2 cases (Table 3). The immunohistochemistry of HIF-1α is demonstrated in figure 1. As the distribution of the two groups was irregular, the Mann-Whitney test was utilized to compare HIF-1α expression. No substantial variance was detected in HIF-1α expression between the two tumor categories (p = 0.578).

Table 1. Samples Characteristics.

Characteristics	Frequency (%)
Gender	
Female	14 (32.55)
Male	29 (67.44)
Age (Years)	
19-30	3 (6.97)
31-40	11 (25.58)
41-50	12 (27.90)
>50	17 (39.53)
Tumor type	
Astrocytoma IDH mutant grade 4	16 (37.20)
Glioblastoma IDH wildtype grade 4	27(62.79)
Total	43 (100)

Table 2. Samples Characteristics based on IDH status.

Tumor type	Gender (%)		Age (%)			
	Female	Male	19-30	31-40	41-50	>50
Astrocytoma IDH mutant grade 4	6 (13.95)	10 (23.25)	0 (0)	7 (16.27)	4 (9.30)	5 (11.62)
Glioblastoma IDH wildtype grade 4	8 (18.60)	19 (44.18)	3 (6.97)	4 (9.30)	7 (16.27)	13 (30.23)
Total	14 (32.55)	29 (67.44)	3 (6.97)	11 (25.58)	11 (25.58)	18 (41.86)

Tabel 3. HIF-1α and VEGF expression based on IDH status.

Tumor type	HIF-1α (%)				VEGF (%)			
	0	+1	+2	+3	0	+1	+2	+3
Astrocytoma IDH mutant grade 4	1 (2.32)	12 (27.90)	2 (4.65)	1 (2.32)	0 (0)	4 (9.30)	10 (23.25)	2 (4.65)
Glioblastoma IDH wild type grade 4	1 (2.32)	18 (41.86)	8 (18.60)	0 (0)	0 (0)	11 (25.58)	10 (23.25)	6 (13.95)
Total	2 (4.64)	30 (69.76)	10 (23.25)	1 (2.32)	0 (0)	15 (34.88)	20 (46.51)	8 (18.60)

0: negative; +1: weak; +2: moderate; +3: strong

VEGF expression

The cytoplasm of the tumor cells exhibited VEGF expression. This study revealed VEGF expression was found in all cases, with various IRS scores ranging from weak (34.88%), moderate (46.51%) and strong (18.60%) (Table 3). The immunohistochemistry of VEGF is demonstrated in figure 2. VEGF expression levels in both tumor types were compared using the Mann-Whitney test, revealing no meaningful variance between the groups (p=0.918).

The correlation of HIF-1α and VEGF in both IDH status

Our investigation involved the utilization of the Spearman's correlation test to assess the relationship between HIF-1α and VEGF expression, uncovering a notable association ($p \leq 0.05$) between the two factors in both tumor types.

DISCUSSION

Previous studies found that grade 4 astrocytoma mainly affected males, with male to female ratios varying between 1.2:1 and 2.6:1.[11] However, our study revealed with a ratio of males to females of 2.07:1. Cases were distributed across ages ranging from 19 to 63 years, with the majority of cases falling in the over-50 age bracket, resulting in an average age of 46.81 years. Previous study showed that the most common age for glioblastoma was above 50 years, due to the aging process increasing immunosuppression in the circulation and CNS, which contributes to the development of glioblastoma cells.[12]

The WHO classification divides diffuse astrocytoma based on IDH status. The immunohistochemistry method is acceptable for determining the IDH status in glioma.[4,5] This study discovered that 27 (62.78%) cases were glioblastoma IDH wild type and 16 (37.78%) cases were astrocytoma IDH mutant. These findings were consistent with previous studies, which revealed that glioblastoma IDH wild-type was more common than grade 4 astrocytoma IDH-mutant.[13] In this study, grade 4 astrocytoma IDH mutant was most commonly found in individuals aged 31-40 years, and astrocytoma with wild-type IDH was most common in the older age group. This observation aligns with previous research indicating that the majority of grade 4 astrocytoma IDH mutants were aged 30-40 years, while the majority of glioblastoma IDH wild type were over 55 years old.[14] The presence of IDH mutant is more common in younger ages, yet the correlation between IDH mutation and age has not been understood.[15]

This study found no difference in HIF-1α expression between mutant and wild-type IDH groups. HIF-1α expression and IDH mutation status can be independent prognostic indicators even though there is no direct signaling pathway between them.[16] On the contrary, Yalaza *et al.* (2017) found an increased expression of HIF-1α in glioblastoma with IDH mutation. The decreased levels of α-ketoglutarate (KG) in IDH mutated cells may stabilize HIF-1α via prolyl hydroxylase (PHD) inhibition, promoting tumor progression. Another possible

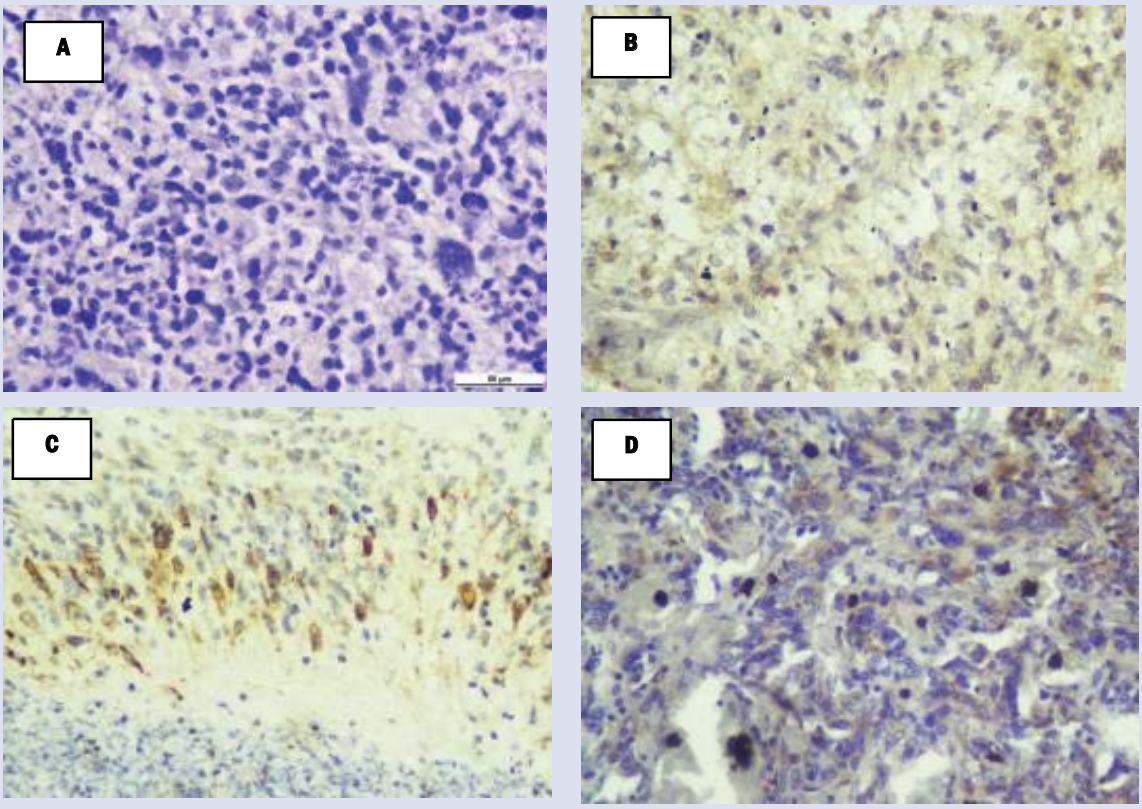

Figure 1. HIF-1α expression in the nuclei of tumor cells (magnification: 400x). A. Tumor cells exhibiting no detectable HIF-1α expression. B. Tumor cells displaying weak HIF-1α expression. C. Tumor cells demonstrating moderate HIF-1α expression. D. Tumor cells with strong HIF-1α expression.

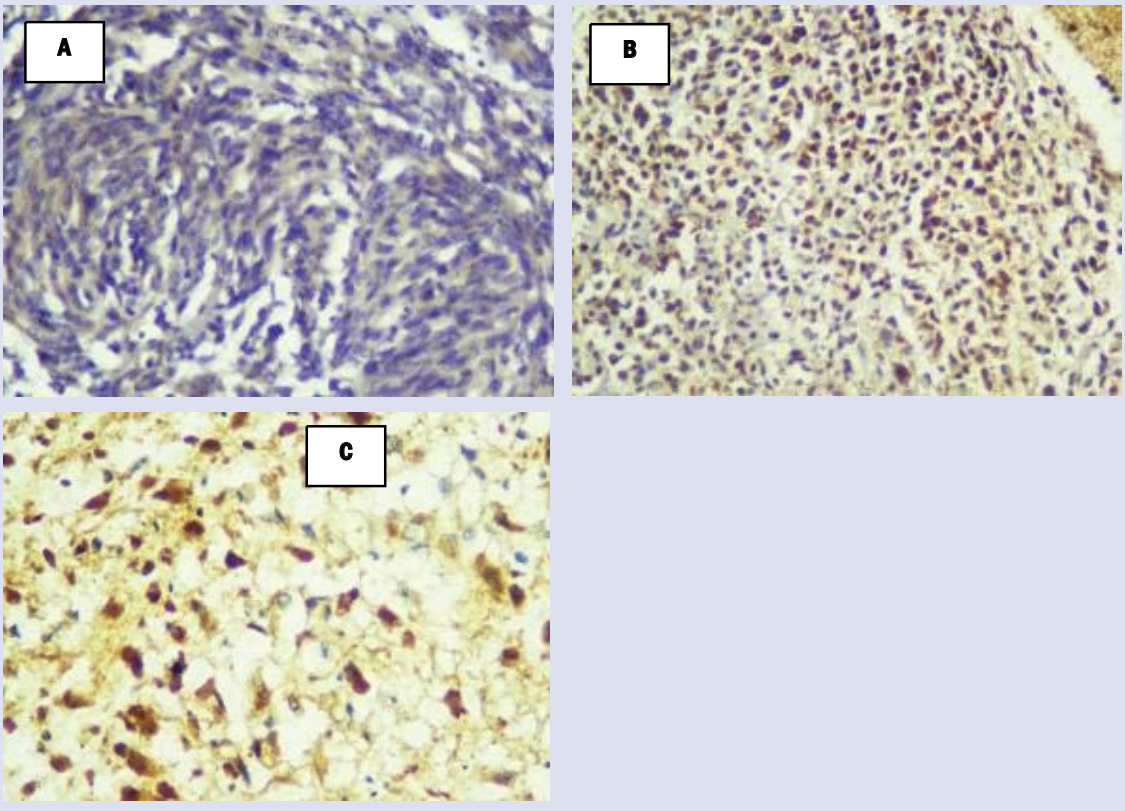

Figure 2. VEGF expression (magnification 400x). A. Tumor cells exhibiting weak expression. B. Tumor cells demonstrating moderate expression. C. Tumor cells displaying strong expression.

explanation is that 2-hydroxyglutarate, an oncometabolite produced in IDH mutated cells, inhibits PHD by competing for α-ketoglutarate binding. Furthermore, 2-hidroxyglutarate (2-HG) inhibits PHD and increases HIF-1α levels in the brain-specific IDH1 R132H condition. It is suggested that IDH mutant may contribute to early carcinogenesis of glioblastoma by inducing the HIF-1α pathway.[17]

VEGF is a protein that holds significance in angiogenesis.[18] Frequently, VEGF is excessively expressed and leads to angiogenesis to provide oxygen and nutrients to the tumor.[19] Several research studies have indicated a connection between elevated VEGF expression and a more aggressive tumor phenotype, leading to increased tumor growth and invasiveness.[20] VEGF expression is generally elevated in glial tumors when compared to normal tissue. However, the expression of VEGF does not seem to differ between high-grade and low-grade gliomas.[21] This study found no different expression of VEGF in IDH type and IDH mutant tumors. Previous studies showed different results, revealing that VEGF expression showed a notable decrease in the mutated IDH and wild-type IDH.[22] In contrast to different signaling pathways of VEGF and IDH, both increasing VEGF expression and mutant type IDH can indicate a worsening prognosis in glioblastoma.

This study revealed the interaction of HIF-1α and VEGF expression within each tumor type. A previous study by Clara *et al.* found a positive association of HIF-1α expression with VEGF in glioblastoma, yet this investigation did not categorize the IDH status.[23] Hypoxia, oxidative stress, pH changes, and growth factors are all known to activate HIF-1α, which is a transcription factor that controls cell responses to hypoxia. Under normoxia or mild hypoxia, HIF-1α binds to the platelet derived growth factor D (PDGFD) proximal promoter and platelet derived growth factor receptor alpha (PDGFRA) intron enhancer in glioblastoma cells. This induces expression and maintains constitutive activation of Akt signaling, increasing HIF-1α protein levels and activity. HIF-1α regulates VEGF expression during angiogenesis in glioblastoma. VEGF is a protein that promotes angiogenesis and is secreted by endothelial cells, to form new blood vessels from preexisting blood vessels. In the context of glioblastoma, VEGF promotes process of generating fresh blood vessels to provide tumor cells with nutrients and oxygen, thereby facilitating survival and aggressive growth. It has been confirmed that VEGF levels rise in glioblastoma, which promotes tumorigenesis and angiogenesis[11]. HIF-1α and VEGF simultaneously work to promote angiogenesis in glioblastoma. HIF-1α and VEGF show a strong correlation in glioblastoma.[24]

CONCLUSION

HIF-1α and VEGF expression did not vary between grade 4 astrocytomas with mutated IDH and wild-type IDH. A positive interaction of HIF-1α and VEGF was found in both tumor types. Therefore, HIF-1α and VEGF likely contribute to the angiogenesis of grade 4 astrocytomas, regardless of the IDH status.

CONFLICTS OF INTEREST

The authors affirmed that there were no conflicts of interest in this study.

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ETHICAL CLEARANCE

This study has obtained ethical clearance from the Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital Surabaya with reference letter number 2020/120/4/II/2023.

AUTHOR CONTRIBUTION

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all aspects of this work.

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